GPO PRICE $ _____

CFSTI PRICE(S) $ _____

Hard copy (HC) 1.00

Microfiche (MF) .50

ff 653 July 65

EARLY EFFECTS OF CORTICOSTERONE ON AMINO ACID INCORPORATION BY RAT LIVER SYSTEMS SUBSEQUENT TO ITS IN VIVO INJECTION[1]

Henry A. Leon

NASA, Ames Research Center
Environmental Biology Division
Moffett Field, California

N66-22216
(ACCESSION NUMBER)

18
(PAGES)

TMX 56676
(NASA CR OR TMX OR AD NUMBER)

1
(THRU)

(CODE)

04
(CATEGORY)

FACILITY FORM 602

Running Head: Early effects of corticosterone

NASA Ames Only

EARLY EFFECTS OF CORTICOSTERONE ON AMINO ACID INCORPORATION BY RAT LIVER SYSTEMS SUBSEQUENT TO ITS IN VIVO INJECTION[1]

Henry A. Leon

NASA, Ames Research Center
Environmental Biology Division
Moffett Field, California

STOCK COPY

Running Head: Early effects of corticosterone

ABSTRACT. Fasted male rats were given single intraperitoneal injections of corticosterone or of saline. Amino acid incorporation into liver microsomal and cell-sap protein was subsequently assayed using cell-free systems. Endogenous pyruvate kinase and added phospho-enol pyruvate were used to maintain nucleotide triphosphate levels necessary for prolonged incorporation. Doses of 25 or 50 μg/Kg were effective in stimulating incorporation. The latter dose significantly stimulated microsomal incorporation 20% at 15 min and 118% at 30 min. Larger doses had no effect at these time intervals. In part, this was attributed to feedback inhibition of the pituitary adrenal axis with a resultant decrease in endogenous corticosterone secretion. Stimulation with a wider range of doses was also seen in adreno-demedullated rats, thus largely excluding epinephrine as a factor. When intact rats were injected with stimulatory doses and subsequently tested in an incorporation system utilizing added creatine kinase and creatine phosphate for energy regeneration, no consistent stimulation of incorporation at 15 or 30 min was observable. The results suggest a rapid effect of corticosterone on endogenous liver pyruvate kinase activity.

INTRODUCTION

It is well established that large or repetitive doses of glucocorticoids can lead to a stimulation of liver protein synthesis (1,2). A degree of specificity is demonstrated in this stimulation since some enzymes concerned with gluconeogenesis are preferentially affected (2,3).

Recent studies directed toward an understanding of the primary action of this class of hormones offer evidence to support a number of views (2,4,5,6,7). Much of the previous work employed either massive doses of corticoids not

necessarily natural to the experimental animal, or steroids altered so as to prolong their action (1,2,6,7). Geller et al. (8) has shown that such administrations give rise to changes not encountered by naturally induced elevations in endogenous corticosteroid levels. Corticosterone is the predominant glucocorticoid in the rat (9). Therefore, it seemed appropriate to study its effect on subsequent in vitro protein synthesis when administered in vivo in quantities compatible with known physiologic levels.

MATERIALS AND METHODS

Male Sprague-Dawley rats were obtained from Simonsen Farms, Gilroy, California. The weights ranged between 220-290 g (7 to 9 weeks old). Adreno-demedullated rats 6 weeks old were obtained from the same source 3 weeks prior to experimentation. Histologic sections of the adrenal glands from these rats showed no evidence of medullary tissue. All animals were maintained on Purina Laboratory Chow in facilities with a 6 AM to 6 PM daylight cycle.

The animals were weighed and fasted 18 hours prior to injection and this weight used to calculate the dose. The corticosterone was suspended in saline by homogenization, usually at a concentration of 50 μg/ml. To minimize the diurnal effect (10), injections were initiated between 9 AM and 11 AM.

Injections were done without noticeably exciting the animals. Controls were injected with saline. The animals were treated and processed at 3-min intervals, usually in groups of 8. They were sacrificed by decapitation and bled for 10 sec. The excised livers were rinsed and homogenized, and post-mitochondrial supernatants were prepared in a manner previously described (1,11). The medium had a final concentration of 0.025M KCl, 0.01M $MgCl_2$, 0.035M Tris buffer (pH 7.8) and 0.25M sucrose. For the incubation, each

tube contained 20 μmoles of tricyclohexylammonium PEP,[2] 2 μmoles disodium ATP, 0.4 μmoles disodium GTP, 0.1 μmoles valine-1-^{14}C (0.588 μc) and 1.4 ml of post-mitochondrial supernatant. The potassium concentration was adjusted to 150 mM with KCl in a final volume of 2.3 ml. In some experiments (Table 1), the valine-1-^{14}C had an activity of 0.625 μc in 0.037 μmoles.

When creatine phosphate was used to regenerate nucleotide triphosphate, 20 μmoles of disodium creatine phosphate and 200 μg (4 enzyme units) of creatine kinase per tube were substituted for the PEP.

The incubation period was 10 minutes at 37 C. The reaction was stopped by placing the tubes in an ice bath and by diluting with cold medium. Microsomes and cell sap were separated by centrifugation; each fraction was precipitated and extracted and the protein residue was pressed onto copper planchets having a diameter of 0.85 cm (1,11). They were counted in a Tracerlab Omni-Guard low background gas flow counter. Activity was expressed as dpm/mg of protein by use of a ^{14}C methacrylate standard for calibration and by employment of the equations for self-absorption corrections suggested by Muramatsu and Busch (12). Sufficient counts were taken to ensure less than 4% counting error with the cell-sap protein and less than 2% counting error with the microsomal proteins.

Corticosterone was estimated on plasma obtained from heparinized blood collected at the time of decapitation. A microfluorometric method was employed for the determinations (13). The values reported are uncorrected for residual plasma fluorescence which averaged 10.6 μg/100 ml of plasma as determined on adrenalectomized rats.

Valine-1-^{14}C was obtained from New England Nuclear Corporation, Waltham, Massachusetts. All other biochemicals and enzymes were obtained from Cal Biochem, Los Angeles, California.

RESULTS

Alterations in Amino Acid Incorporation. In preliminary experiments it was found that 25 or 50 μg/Kg of corticosterone would stimulate incorporation within 15 to 30 min relative to saline-injected controls. After 1 hr a stimulation was not observed. Larger doses were ineffective at these time periods.

The results with pyruvate kinase-dependent systems prepared 30 min following the injection of either saline or corticosterone are shown in Fig. 1. A one-way analysis of variance of the 7 groups indicated a significant difference in the means ($p < 0.025$). In particular, using Scheffe's method of comparison (14), it was found that the group dose of 50 μg/Kg was significantly different from that of the saline-injected group ($p < 0.05$). No other significant contrasts were found.

Incorporation in Adreno-Demedullated Rat Preparations. The use of saline-injected controls largely eliminates epinephrine as a factor in the changes in amino acid incorporation shown in Fig. 1. Nevertheless, since this hormone is also reported to affect the rate of liver protein synthesis (15), its possible involvement was further tested by making use of adreno-demedullated rats.

Amino acid incorporation was tested 30 min following the injection of saline or of corticosterone in doses ranging from 25 to 250 μg/Kg. The results are illustrated in Fig. 2. Incorporation into microsomal protein was significantly elevated at all dose levels tested. A two-way analysis of variance was performed on microsomes, with dosage and blocks (experiment number) as the two factors. It was found that the dose response was significantly greater ($p < 0.025$) than the control group for all levels of doses. No significant differences between dose levels were found. A similar analysis of cell-sap response showed no statistically significant changes. With both intact and adreno-demedullated rats, however, the parallelism between microsomal and cell-sap incorporation rates is nevertheless apparent.

Involvement of the Energy Regeneration System. In the experiments so far described, continued incorporation was facilitated by using endogenous pyruvate kinase to regenerate nucleotide triphosphates from added phospho-enol pyruvate. Therefore, the observed changes could be due to alterations in the activity of this enzyme through its capacity to maintain the energy level of the system. This possibility was tested by simultaneously comparing incorporation from saline- and corticosterone-injected rats with both the above system and one which depends on added creatine phosphate and creatine kinase for optimal activity.

In preliminary experiments it was found that 2 to 4 enzyme units of creatine kinase caused optimal activity when the PEP was replaced by an equivalent amount of creatine phosphate. The level of incorporation is considerably higher than in a comparable PEP-dependent system; yet the creatine phosphate system is capable of accommodating even greater rates of incorporation, for example, when liver preparations from fed rats or from stress-stimulated rats are employed (11). Therefore, the rate of nucleotide triphosphate regeneration is not the limiting factor in this creatine phosphate-dependent system, whereas this could be the case when incorporation is dependent on endogenous pyruvate kinase levels. Thus, if an observed stimulation by corticosterone is due to factors other than a more efficient energy regeneration, both systems should show the increase.

Such a comparison of preparations obtained 15 min after the in vivo injection of either saline or of 50 μg/Kg of corticosterone is shown in Table 1. The comparison shows that corticosterone significantly stimulates incorporation into microsomal protein in the pyruvate kinase system by about 20% ($p < 0.05$), whereas in the creatine kinase system no appreciable change is detected. In both cases cell-sap incorporation is stimulated, but in neither case is it statistically significant. In experiments not shown here it was not possible to show a stimulation at 30 min with the creatine kinase system.

Changes in Plasma Corticosterone. With the aim of relating the aforementioned changes in amino acid incorporation to alterations in plasma corticosterone levels, such determinations were made on plasma obtained 0.5 hr following the injection of the various doses. These data, recorded in Fig. 3, show that only doses of 1000 μg/Kg or greater result in plasma elevations significantly greater ($p < 0.001$) than the saline-injected controls. With the effective dose of 50 μg/Kg, a slight but nonsignificant increase was observed. Unexpectedly, intermediate doses caused depressed plasma levels. Thus, at a dose of 200 μg/Kg, plasma corticosterone levels are approximately 12 μg% lower than at a dose of 50 μg/Kg, This effect appears to be due to a rebound phenomenon since significant increases with these doses occur 8 min following their injection.

It should also be pointed out that initial plasma levels here, being on the order of 42 μg%, are somewhat higher than those reported by others (16); however, in the present work fasted rats were employed. Determinations made with fed rats gave values compatible with the previously reported figures. This elevated fasting level, nevertheless, probably serves to make any tendency to rebound more evident.

Changes in plasma corticosterone levels at times following the injection of a set dose of 50 μg/Kg or of saline were also evaluated. The results are shown in Fig. 4. A one-way analysis of variance performed on these data showed a significant difference in the means ($p < 0.001$). In particular, 15 min following the injection, the corticosterone-injected group showed a significant rise above both the saline-injected controls and the noninjected group ($p < 0.05$). The tendency to rebound for both injected groups was also noted here but was delayed somewhat.

DISCUSSION

Interpretation of the amino acid incorporation data, to a large extent, depends on a knowledge of the plasma changes in corticosterone subsequent to its injection. A considerable amount of evidence indicates that these levels are controlled by a negative feedback system; and the changes described in Figs. 3 and 4 are compatible with this contention (17). For example, it is known that ACTH release is inhibited by increased plasma levels of numerous corticoids. Such an action by corticosterone is an explicit requirement if its plasma level is to be controlled by such a system (17,18). Also, since circulating corticosterone has a rather short half-life, being on the order of 15-25 min (19), it is probable that above a certain threshold, the rapid rise in plasma levels subsequent to its administration could so depress the release of ACTH, and consequently endogenous corticosterone production, that the decay process would momentarily predominate. On the other hand, when larger doses of corticosterone are administered, the amounts given could be sufficiently large to counter the decay process for extended periods.

Thus, Harding et al. (20) suggested that the inhibition of liver alanine transaminase by the mineralo-corticoid, deoxycorticosterone is caused by such a suppressive action on ACTH release. Ordinarily this enzyme is maintained by adrenal gluco-corticoids; however, they found that deoxycorticosterone was without effect in hypophysectomized rats, nor did it prevent increases in activity induced by ACTH administration to such rats. Likewise, it was found that a number of corticoids including deoxycorticosterone generally had a depressant effect on amino acid incorporation into liver systems subsequent to the in vivo injection of an intermediate range of doses (1).

Thus, it would seem that modest increases in plasma corticosterone can lead to a stimulation of pyruvate-kinase-dependent amino acid incorporation above the basal level. Larger unphysiologic amounts neither stimulate nor depress incorporation significantly for the time intervals studied here. The reasons for this are not entirely clear.

The stimulation occurs 15 min after corticosterone injection. Shorter intervals were not tested. According to the work of Hodges and Jones (16), peak plasma levels of corticosterone injected in a fashion similar to that employed in the present work occur at about 7 min. Therefore, the effect of this corticoid on pyruvate-kinase-dependent incorporation would appear to be rather direct and rapid. The fact that stimulation is still evident 30 min after injection when plasma levels are normal or nearly so is not contradictory to this idea since tissue levels may still be elevated.

Whether the first action of corticosterone is on pre-existing pyruvate kinase or whether it acts by some other means cannot be stated on the basis of presently available information since early changes in messenger RNA have been reported with massive doses of cortisol, and it is possible that corticosterone might have a similar action (6). However, the data as presented here would give more credence to an activation.

Nonetheless, it is interesting that present concepts concerned with the gluconeogenic activity of gluco-corticoids give great importance to the formation of phospho-enol pyruvate from lactate and pyruvate (21). Presumably, this is controlled, in part, through the action of pyruvate carboxylase which is activated by acetyl coenzyme A (22). Thus, one possibility considered by others (21,22,23) is that within the structured order of the cell, increased pyruvate kinase activity could lead to increased levels of acetyl coenzyme A and thereby stimulate the process of gluconeogenesis.

ACKNOWLEDGMENTS

The author wishes to thank B. Daligcon and M. Rowley for technical assistance, and E. Averkin for assistance in the statistical evaluation.

TABLE 1. *In vitro* amino acid incorporation 15 min subsequent to *in vivo* injection of 50 μg/Kg of corticosterone or of saline vehicle. Animals were simultaneously tested in two differing incorporation systems. In one case, PEP and endogenous pyruvate kinase were used. In the other, incorporation was dependent on added creatine kinase and creatine phosphate. The 1-^{14}C valine had an activity of 0.625 μc in 0.037 μmoles. Values are given as in Fig. 1. Each group consisted of 8 animals.

	Saline	Corticosterone	p
PEP microsomes	1547±79	1846±91	< 0.05
Creatine phosphate microsomes	2503±121	2579±225	NS
PEP cell sap	112±6	134±15	NS
Creatine phosphate cell sap	226±13	270±32	NS

NS = not significant.

FOOTNOTES

[1]Portions of this paper were presented at the 49th Annual Meeting of the Federation of American Societies for Experimental Biology, Atlantic City, New Jersey, April 13, 1965.

[2]Abbreviations: PEP, phospho-enol pyruvate; PEP.TCHA, tricyclohexyl ammonium salt.

REFERENCES

1. Leon, H. A., E. Arrhenius, and T. Hultin, Biochim Biophys Acta 63:423, 1962.
2. Weber, G., R. L. Singhal, N. B. Stamm, E. A. Fisher, and M. A. Mentendiek, In Weber, G. (ed.), Advances in Enzyme Regulation, MacMillan, New York, 1964, vol. 2, p. 1.
3. Rosen, F., and C. A. Nichol, In Weber, G. (ed.), Advances in Enzyme Regulation, MacMillan, New York, 1964, vol. 2, p. 115.
4. Knox, W. E., In Weber, G. (ed.), Advances in Enzyme Regulation, MacMillan New York, 1964, vol. 2, p. 311.
5. Ray, P. D., D. O. Foster, and H. A. Lardy, J Biol Chem 239:3396, 1964.
6. Kidson, C., and K. S. Kirby, Nature 203:599, 1964.
7. Kenney, F. T., and F. J. Kull, Proc N. A. S. 50:493, 1963.
8. Geller, E., A. Yuwiler, and S. Schapiro, Biochim Biophys Acta 93:311, 1964.
9. Peron, F. G., Endocrinology 66:458, 1960.
10. Critchlow, V., R. A. Liebelt, M. Bar-sela, W. Mountcastle, and H. S. Lipscomb, Amer J Physiol 205:807, 1963.
11. Leon, H. A., D. D. Feller, E. Neville, and B. Daligcon, Life Sci 4:737, 1965.
12. Muramatsu, M., and H. Busch, Anal Biochem 4:384, 1962.
13. Wherry, R. E., L. N. Trigg, R. E. Grindeland, and E. Anderson, Proc Soc Exp Biol Med 110:362, 1962.
14. Scheffe, H., The Analysis of Variance, Wiley and Sons, Inc., New York, 1959.
15. Henriques, S. B., O. B. Henriques, and F. R. Mandelbaum, Biochem J 66:222, 1957.
16. Hodges, J. R., and M. T. Jones, Physiol 167:30, 1963.
17. Yates, F. E., and J. Urquhart, Physiol Rev 42:359, 1962.

18. Martini, L., M. Fochi, G. Gavazzi, and A. Pecile, Arch Int Pharmacodyn 40:156, 1962.

19. Glenister, D. W., and F. E. Yates, Endocrinology 68:747, 1961.

20. Harding, H. R., F. Rosen, and C. A. Nichol, Proc Soc Exp Biol Med 108:96, 1961.

21. Utter, M. F., D. B. Keech, and M. C. Scrutton, In Weber, G. (ed.), Advances in Enzyme Regulation, MacMillan, New York, 1964, vol. 2, p. 49.

22. Lardy, H. A., D. O. Foster, E. Shrago, and P. D. Ray, In Weber, G. (ed.), Advances in Enzyme Regulation, MacMillan, New York, 1964, vol. 2, p. 39.

23. Krebs, H. A., E. A. Newsholme, R. Speake, T. Gascoyne, and P. Lund, In Weber, G. (ed.), Advances in Enzyme Regulation, MacMillan, New York, 1964, vol. 2, p. 71.

FIGURE LEGENDS

FIG. 1. Incorporation of 1-^{14}C valine into protein by cell-free rat liver systems prepared 30 min subsequent to the in vivo injection of various doses of corticosterone or saline. A PEP.TCHA system using 0.588 μc in 0.1 μmole of 1-^{14}C valine was employed. (See methods.) Abscissa: dose of corticosterone. Broken line: microsomal protein specific activity with values on left ordinate. Solid line: cell-sap protein specific activity with values on right ordinate. The number of animals tested at each point is shown in brackets. Standard errors are indicated.

FIG. 2. Incorporation of 1-^{14}C valine into protein by cell-free rat liver systems prepared 30 min subsequent to injecting adreno-demedullated rats with various doses of corticosterone. Eight animals were used at each point. All other conditions are as indicated in Fig. 1.

FIG. 3. Plasma corticosterone levels from blood obtained 30 min following the intraperitoneal injection of various doses. Abscissa: injected dose in μg/Kg. Ordinate: plasma level in μg/100 ml. Values are uncorrected for background fluorescence. Other conditions as in Fig. 1. Number of animals indicated in brackets.

FIG. 4. Plasma corticosterone levels at time intervals following the intraperitoneal injection of 50 μg/Kg of corticosterone or 0.5 ml of saline vehicle. Zero time represents noninjected animals. Abscissa: Sampling time subsequent to injection. Ordinate: plasma level in μg/100 ml. Number of animals indicated in brackets.

A-34254-1

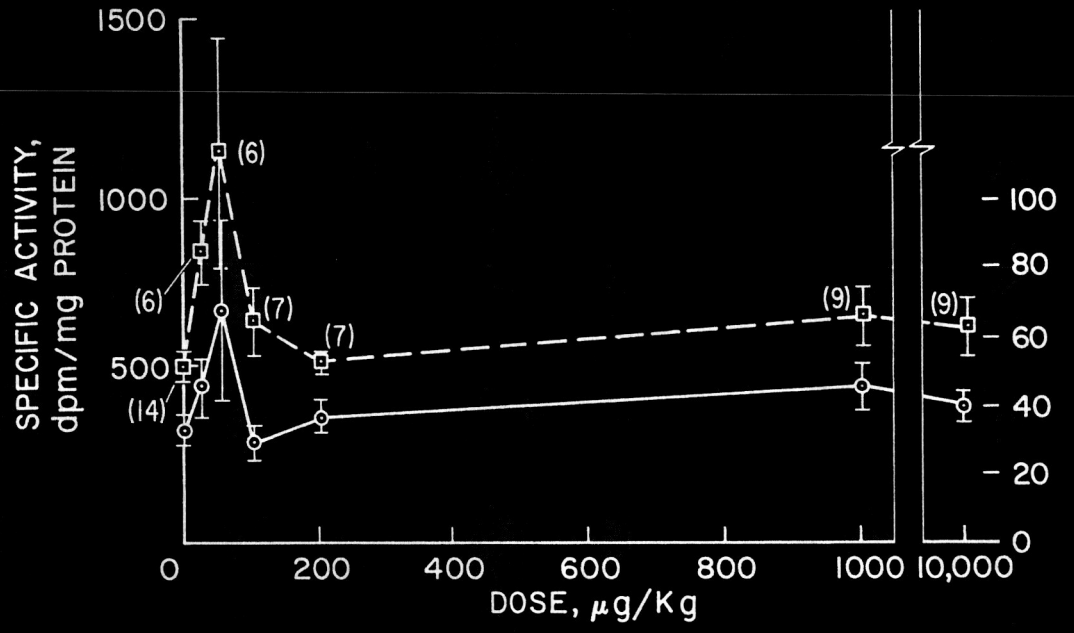
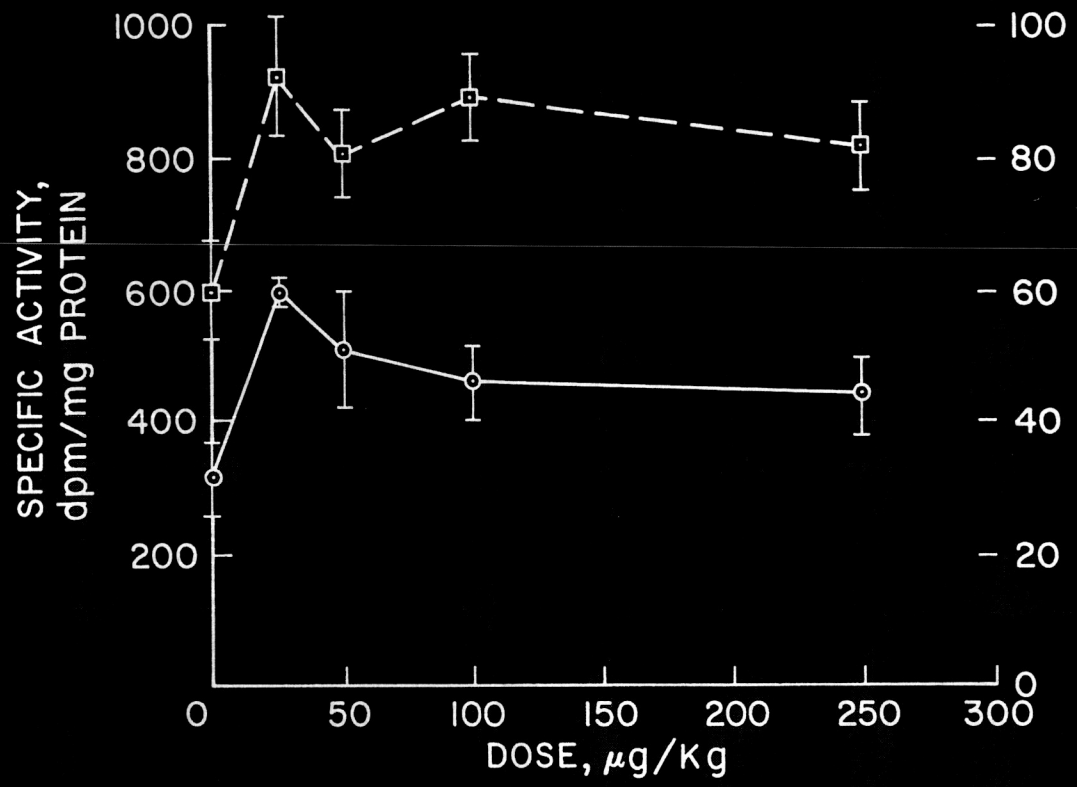


Fig. 1

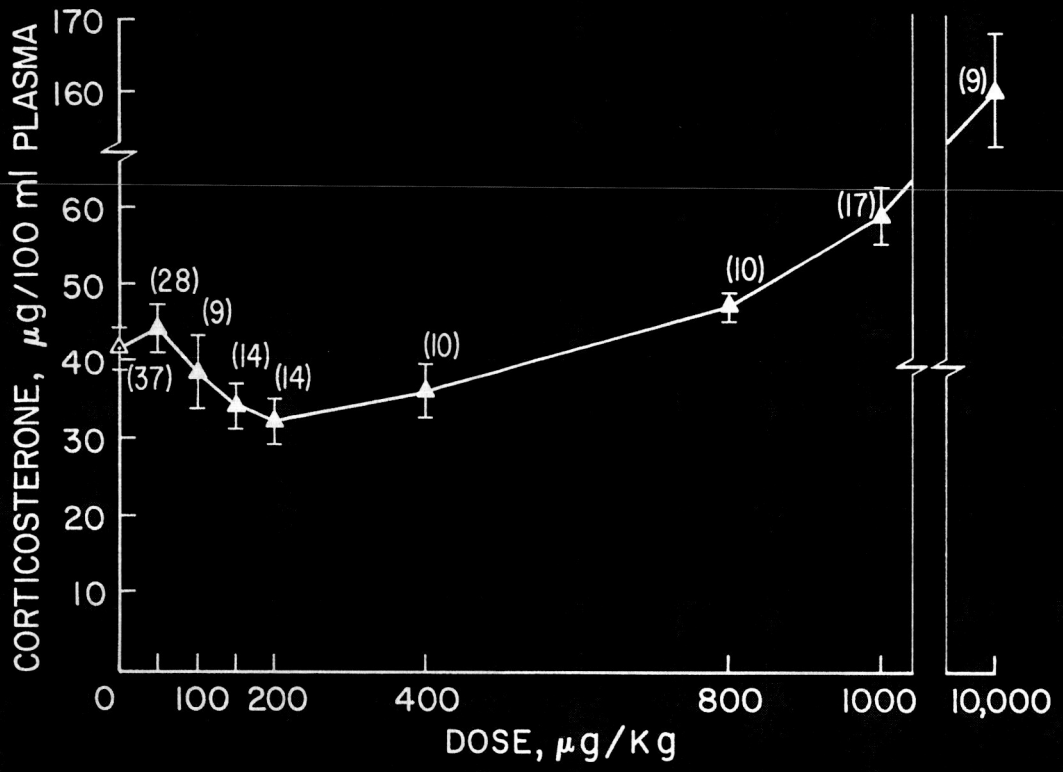
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
AMES RESEARCH CENTER, MOFFETT FIELD, CALIFORNIA



A-34254-2

Fig. 2

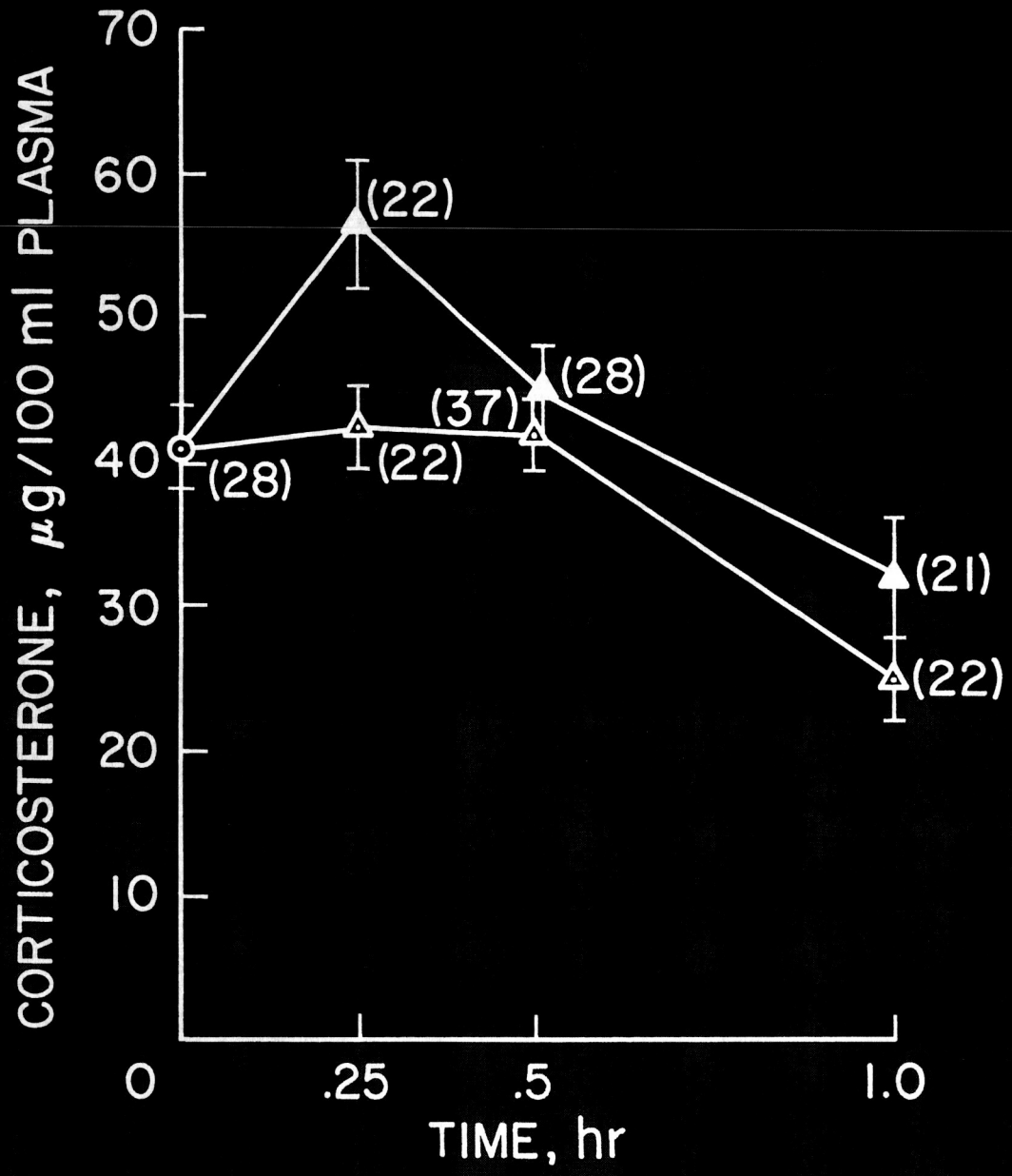
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
AMES RESEARCH CENTER, MOFFETT FIELD, CALIFORNIA



A-34254-3

Fig. 3

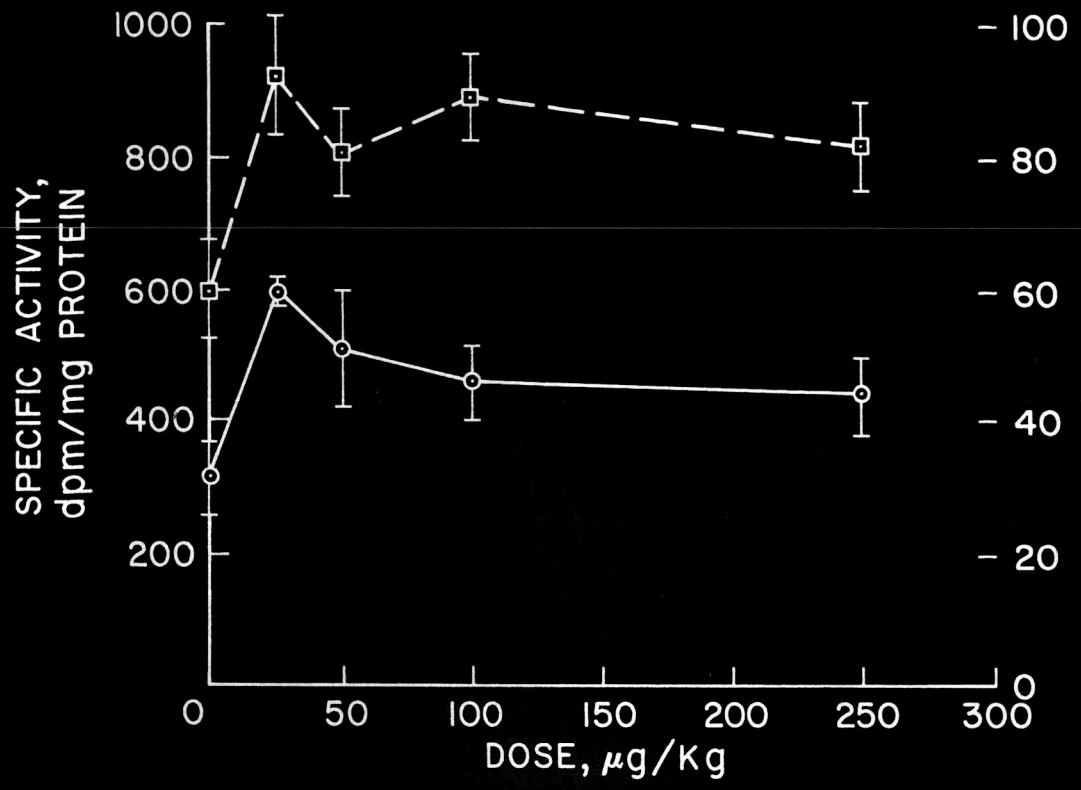
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
AMES RESEARCH CENTER, MOFFETT FIELD, CALIFORNIA



A-34254-4

Fig. 1

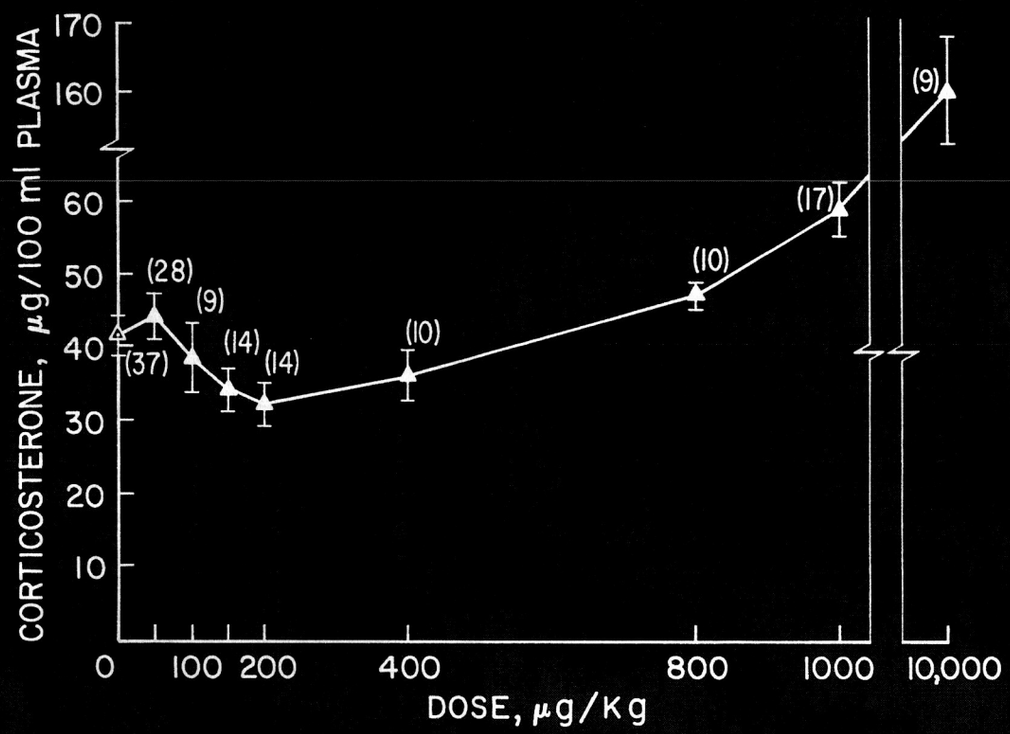
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
AMES RESEARCH CENTER, MOFFETT FIELD, CALIFORNIA



A-34254-2

Fig. 2

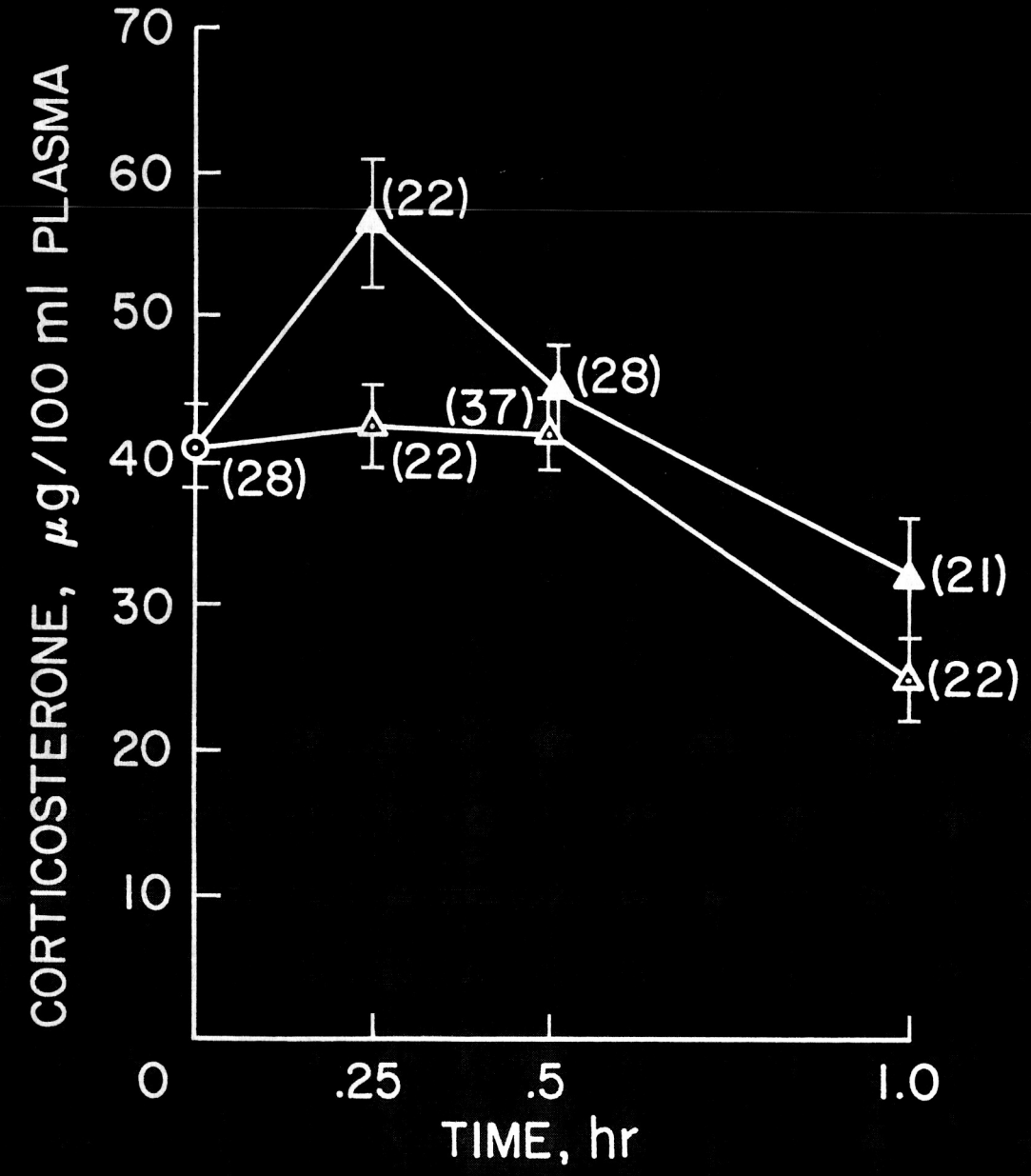
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
AMES RESEARCH CENTER, MOFFETT FIELD, CALIFORNIA



A-34254-3

Fig. 3

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
AMES RESEARCH CENTER, MOFFETT FIELD, CALIFORNIA



A-34254-4

Fig 4

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
AMES RESEARCH CENTER, MOFFETT FIELD, CALIFORNIA